

Article

Exploring Potential of Seed Endophytic Bacteria for Enhancing Drought Stress Resilience in Maize (*Zea mays* L.)

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Abstract: Water scarcity is abiotic stress that is becoming more prevalent as a result of human activities, posing a threat to agriculture and food security. Recently, endophytic bacteria have been proven to reduce drought stress and increase crop productivity. Here, we explored the efficacy of seed endophytic bacteria in maize crops under water deficit conditions. For this purpose, twenty-seven endophytic bacteria have been isolated from three distinct maize cultivars seeds (Malka 2016, Sahiwal Gold and Gohar-19) and evaluated for desiccation tolerance of -0.18 , -0.491 , and -1.025 MPa induced by polyethylene glycol (PEG) 6000. The nine isolates were chosen on the basis of desiccation tolerance and evaluated for maize growth promotion and antioxidant activity under normal and drought conditions. Results showed that drought stress significantly decreased the growth of maize seedlings. However, isolates SM1, SM4, SM19, and SM23 significantly improved the root and shoot length, plant biomass, leaf area, proline content, sugar, and protein content under normal and drought conditions. Antioxidant enzymes were significantly decreased at p -value < 0.05 with inoculation of seed endophytic bacteria under drought conditions. However, further experiments of seed endophytic bacteria (SM1, SM4, SM19, and SM23) should be conducted to validate results.

Keywords: endophytes; seed; drought



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1. Introduction

Plants are continuously confronted with a series of abiotic stresses associated with changing climate that can adversely affect plant productivity. Drought is one of the most damaging abiotic stresses in agricultural production, threatening global food security [1,2]. It is predicted that if this situation persists, 30% of water resources will decline and drought areas will be expected to double by 2050 [3–5]. Literature indicates frequent hot droughts in arid and semi-arid regions which will influence agricultural productivity [6,7].

Drought can cause considerable changes in soil properties, limit nutrient mobility, and decrease microbial decomposition processes in soil [8,9]. Drought plants suffer from low efficiency of water and nutrient absorption, hormonal imbalance, accumulation of Reactive oxygen species (ROS), and reduced photosynthesis [10]. Plants initiate several coping strategies to trigger signaling responses under drought spells [11]. Published research from 1980 to 2015 has shown that drought stresses have generally accounted for up to a 21% decrease in wheat production, and 40% of maize production worldwide [12].

Various strategies, such as selection of tolerant varieties, molecular breeding, and genetic engineering are being used to counter the deleterious effects of water deficit conditions in plants [13]. However, the majority of these methods are time-consuming, costly, and not well accepted in some areas [14]. So far as the increased productivity of crops is concerned, under various abiotic stresses, the methods using various microbial strains from different resources have recently been highlighted as an emerging and revolutionary method of plant growth promotion along with agricultural productivity. During drought,

plant growth promoting Rhizobacteria (PGPR) can directly mitigate the oxidative effect by producing antioxidant and ROS-degrading enzymes in plants under field conditions [15]. Efforts to introduce beneficial bacteria into the rhizosphere of crops have generally failed in multiple ways, mainly because of the difficulty of integrating exotic species into acclimated and established microbial communities. Recently, endophytes have gained interest due to their close accessibility to host plants and their relative protection from environmental extremes i.e., high salt and drought as compared to soil bacteria [16,17].

Endophytes are a class of endo-symbiotic microorganisms that live inside the plant tissues [18]. While observing plant-endophyte interaction, endophytes obtain carbohydrates and in return, they benefit plants for improving stress tolerance [19]. Endophytes colonize apoplasts of plants, spaces present between the cell wall and root-xylem channels, stems, leaves, flowers, fruits, and seeds of the plant [20]. Endophytes have been shown to help plants respond to stressful environments by the exchange of essential nutrients and enzymes, and the production of secondary metabolites [21,22]. Endophytes can produce phytohormones such as cytokinin [23] and indole acetic acid (IAA) [24,25].

Maize (*Zea mays* L.) is a widely grown staple grain of global dietary importance, staving off hunger for billions of people [26]. It is a C4 crop, a cultigen of global economic importance [27]. Data clustered on maize production reveals that Pakistan ranked 29th in total maize production among the leading maize-growing countries in the world [28]. Climate induced stresses have disastrous consequences on maize production and yield. The disruption of the agri-food system is escalating the issues of food insecurity [29]. Maize is a plant that is often dehydrated during its vegetative and reproductive stages, affecting its growth and grain yield [30]. Annual yield losses due to drought average around 15% of potential yield [31].

It has been reported that seeds contain a diverse microbial community and they can quickly colonize in the new seedlings [32]. The recent literature has revealed the role of seed endophytic bacteria for plant growth promotion [33–35]. The knowledge of seed endophytic bacteria for stress tolerance is still scarce. We hypothesized that endophytic bacteria inside a dry seed may have better prospects to improve maize growth under drought stress. Thus, the present study was aimed at isolating different drought tolerant endophytic bacteria from maize seeds. In addition, the potential of seed endophytic bacteria for improving the growth, physiology, and antioxidant activity of maize under drought stress was also explored.

2. Materials and Methods

1. Isolation of seed endophytic bacteria from maize seeds

Healthy seeds of three maize (*Zea mays* L.) cultivars (Malka 2016, Sahiwal Gold and Gohar-19) were collected from Ayub Agricultural Research Institute, Faisalabad-Pakistan and used for endophytic bacteria isolation by a dilution and plating technique. Surface sterilization of seeds was accomplished by immersing them in 70% ethanol for 60 s, 5% sodium hypochlorite (NaClO) for 3 min, and rinsing five times with sterilized distilled water. To check the sterilization efficiency one ml aliquot of the last rinse was plated on Luria Bertini (LB) agar plates. The surface sterilized seeds were triturated moderately and serially diluted in 0.85% Sodium chloride solution (NaCl) [36]. The sample from appropriate dilution was plated on LB agar plates and placed in an incubator at 28 ± 1 °C for 3–5 days. The morphologically distinct bacterial colonies were purified and stored in permanent glycerol stock at -80 °C for further use.

2. In-vitro screening of isolates for drought tolerance

Twenty-seven bacterial isolates were screened for desiccation tolerance using Poly Ethylene Glycol (PEG). Tryptic soy broth (TSB) was supplemented with no PEG, 10%, 20%, 30% PEG-6000 to provide an osmotic potential of 0, -0.18 , -0.491 , and -1.025 MPa, respectively, measured by Cryoscopic Osmometer (OSMOMAT-030-D, Gonotec, Germany) and inoculated with an overnight raised culture of bacterial isolates. After 48 h of incubation at 28 °C under shaking conditions, drought tolerance was assessed by measuring

optical density at 600 nm [37]. The optical density of seed endophytic bacterial strain at different drought levels (0%, 10%, 20% and 30%) was standardized by means and Euclidean distances for similarities in the variables were calculated. The ward method was applied to standardized data for hierarchical clustering.

3. Phenotypic and biochemical characterization of drought tolerant endophytic bacteria

The Gram reaction was performed on nine pure drought tolerant isolates, which were subsequently subjected to biochemical assays and morphological characteristics. The ability of isolates to grow on different salt concentrations was investigated by streaking isolates on plates containing LB media with NaCl (0.5%, 1%, 1.5%, 2%, and 2.5% *w/v*) [38]. The isolates were also tested at different pH levels (4, 5, 6, 7, 8, and 9) [39].

The drought tolerant isolates were further tested for plant growth promoting attributes *in vitro*. For the determination of phosphate solubilization, a freshly prepared culture of each isolate (10 μ L) was inoculated at four different points of Pikoviskaya's media plates. The plates were incubated for seven days at 30 °C. Phosphorus (P) solubilization was assessed by checking developed clear zones created around the bacterial colonies [40]. For evaluation of siderophore production, Chrom azurol S (CAS) agar containing plates were inoculated with a freshly prepared 5 μ L culture of 0.5 OD. Then plates were kept in an incubator for 48 h at 28 ± 1 °C. Plates with a change in media colour from blue to orange were considered positive for siderophore [41]. The auxin production in the presence and absence of L-tryptophan (L-TRP) was measured using a spectrophotometer at a wavelength of 535 nm in terms of indole acetic acid (IAA) equivalent. IAA concentration was calculated by using appropriate standards [42]. The 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity was quantitatively determined by method described by Penros and Glick [43]. The isolates were tested for catalase by following Chester procedure [44]. For this purpose, a smear of bacterial colonies was put on a glass slide and hydrogen peroxide (H₂O₂) 35% was dropped with the help of a dropper on colonies. The production of bubbles in response to H₂O₂ dropping was a positive sign for catalase presence. For exopolysaccharide production, the RCV-glucose media plates were inoculated at four different positions by loopful of inoculum and placed in an incubator for 96 h. After incubation, the plates showing mucoid growth around colonies were supposed to be positive [45]. The ability to degrade chitin was assessed by inoculating a loopful of inoculum on LB agar plates containing chitin [46]. The presence of halos zones around the colonies was checked after 96 h incubation at 28 ± 1 °C. The colonies showing halos zones were positive for chitinase. The Oxidase activity was observed by rubbing a loopful of bacteria on filter paper containing 1% Kovács reagent [47]. The change in colour of the paper from blue to purple within 90 s indicated oxidase activity.

4. Experimental growth conditions

The growth room experiment was conducted to find the effective bacteria selected from the drought tolerance assay for plant growth promotion at different moisture levels. The seeds of maize (Malka 2016) were surface sterilized as described above. For seed inoculation, endophytic bacterial culture was prepared in Lb broth media for 12 h and then maize seeds were soaked for 4 h before being planted in pots. The pots contained 1 kg autoclaved mixture of soil and sand (3:1). At an average temperature of 25 ± 2 °C, pots were put in a controlled growth room with an artificially induced photoperiod (14 h light and 10 h dark cycle). After 10 days of germination, the seedlings were exposed to drought stress by cutting off the water supply, while the non-stressed plants were kept well-watered. The seedlings were harvested after 21 days and growth, biochemical and antioxidant were measured by following standard protocols.

5. Agronomic parameters

The average of three plants was taken from each replicate to measure plant height and root length using a measuring tape. Plant fresh and dry biomass was also recorded using a laboratory scale.

6. Estimation of biochemical parameters

For proline estimation, the repaid calorimetric method was used as described by [48]. The fresh leaf sample was homogenized with 3% sulphosalicylic acid. Glacial acetic acid and ninhydrine were used to treat the filtrate. After that, samples were placed in the water bath for 1 h at 100 °C and the process was stopped by switching to an ice bath. Toluene was added to the reaction mixture in the last step for extraction, and the absorbance at 520 nm was measured. Known proline standards were used to calculate proline concentration using a standard curve. The soluble sugar was determined by the anthrone reagent method [49]. The sample mixture was prepared by adding 8 mL of 0.2% anthrone (prepared in 95% sulphuric acid) in 2 mL of diluted plant sample. The mixture was heated for 8 minutes. After cooling, absorbance was recorded at 630 nm on the spectrometer. Glucose standards solution was prepared for plotting the calibration curve. The total protein content was measured using the very fast Bradford process. The 200 µL extract of the plant leave sample was taken and further diluted 10 times with distilled water. Then 2 mL of Bradford reagents were mixed into the samples and absorbance was recorded at 595 nm after 20 min. Bovine serum albumin was used for plotting calibration curve to calculate protein content [50].

7. Estimation of Antioxidants activity

The enzymatic extract of plant sample prepared in 0.2 M potassium phosphate buffer was used for estimation of catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR) and superoxide dismutase (SOD) activity. For CAT production, the reaction mixture was prepared by adding 10 mM H₂O₂ in an enzymatic plant extract (200 times diluted with 50 mM potassium phosphate buffer). Using a UV Visible spectrophotometer, the decrease in absorbance was measured for 3 min at 240 nm. The 40 mM⁻¹ cm⁻¹ extinction coefficient for H₂O₂ was used to calculate CAT activity [51]. The GPX activity was calculated by detecting a decrease in absorbance at 340 nm produced by the oxidation of NADPH to NADP⁺ by using a GPX Cellular Activity Assay Kit (Catalog Number CGP1, Sigma, Saint Louis, MO, USA). The enzyme activity was then estimated using 6.22 mM⁻¹ cm⁻¹ extinction coefficient for NADPH [52]. The activity of GR was evaluated by observing an increase in absorbance at 412 nm caused by the reduction of DTNB (5,5-dithiobis (2-nitrobenzoic acid)) by using a glutathione reductase assay kit (Cat. No. GRSA, Sigma, Saint Louis, MO, USA). The extinction coefficient of DTNB (14.15 mM⁻¹ cm⁻¹) was used to measure enzyme activity at 25 ± 2 °C, and the GR activity was expressed in nmol TNB min⁻¹ g⁻¹ fresh weight [53]. The SOD was measured as demonstrated by Roth and Gilbert [54]. The 1 mL reaction mixture was prepared by mixing 100 mM EDTA, 50 mM sodium phosphate buffer (pH 7.8), 10 mM pyrogallol. The SOD content (nmol min⁻¹ g⁻¹ protein) was estimated by using a spectrophotometer at 420 nm wavelength for 120 s at 30 s interval.

8. Statistical analysis

The data of growth room experiment was subjected to analysis through SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). The results were evaluated by Two-Way ANOVA followed by a classification of means with the least significant differences test ($p < 0.05$) to compare the treatments with the control (uninoculated plants).

3. Results

Twenty-seven bacterial strains were isolated from three different maize varieties by dilution and plating techniques [36]. All bacterial isolates were streaked on LB agar plates and tested for drought tolerance ability. The bacterial strains in clusters 1 and 2 showed strong similarity and they are the most drought tolerant as compared to strains in cluster 3. The most efficient strains for drought tolerance were located on the left and lowest on the right side of the dendrogram (Figure 1).

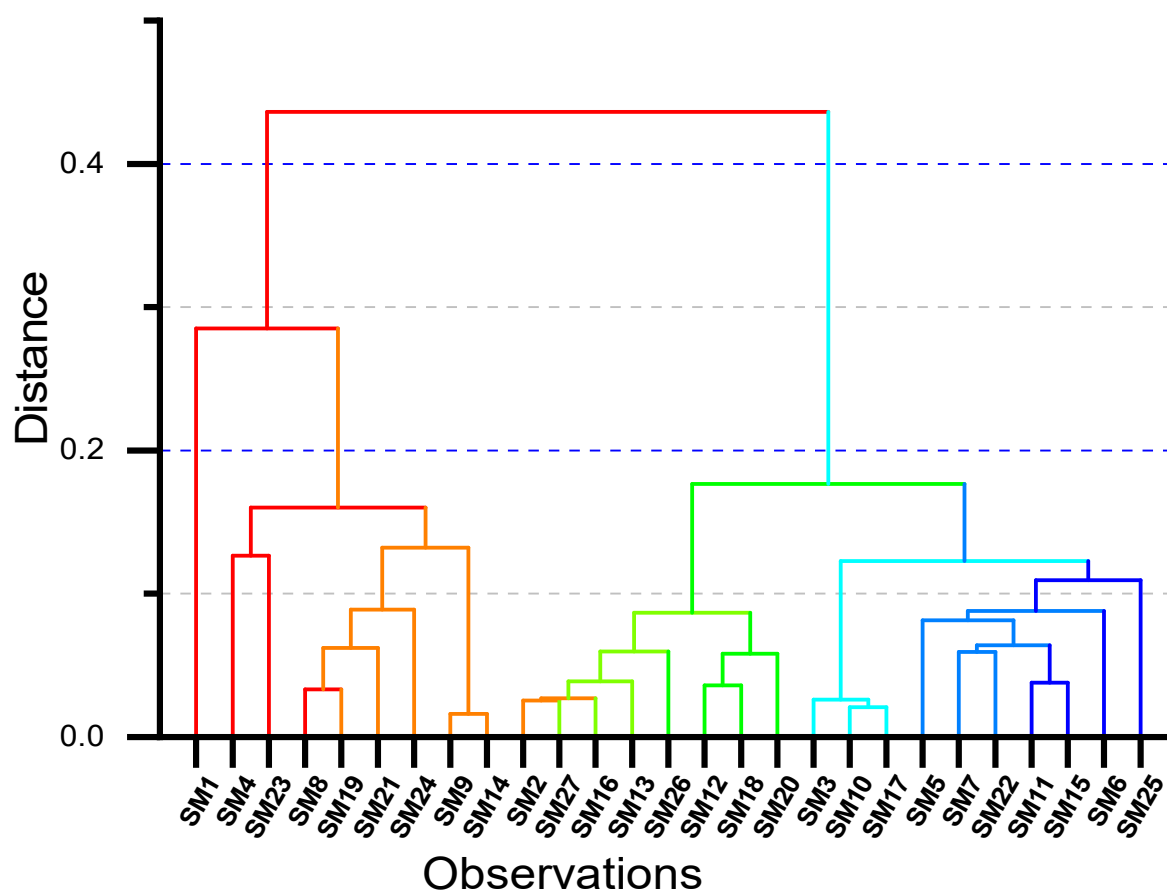


Figure 1. Cluster analysis of seed endophytic bacterial isolates on the basis of similarity index between optical densities (OD) at different drought levels (0, 10, 20 and 30% PEG). Data is the average of three replicates.

The general microscopic view showed that bacterial colonies were rod-shaped and gram-negative. The colonies of all isolates were circular, 1.8–4 mm, yellow and milky white and transparent (Table 1). It has been revealed that seed endophytic isolates can grow under high salt conditions. All the isolates exhibited growth at 0.5–2.5% (*w/v*) sodium chloride stress. The pH values 6 to 7 were found optimum for the growth of endophytic bacteria. However, at pH 4 minimum growth was shown.

Data presented in Table 1 showed the characterization of selected seed endophytic bacteria. It was clear from the results that 7/9 for P solubilization, 4/9 for siderophore, 7/9 for oxidase, and 6/9 for chitinase were found positive by showing clear zone on their respective media. All the isolates were positive for catalase activity. The 7/9 were found positive for exopolysaccharide production by showing mucoid growth around the colonies. These isolates were also confirmed for ACC deaminase activity and IAA production quantitatively. The isolates produced IAA in the range of 25 to 45 $\mu\text{g mL}^{-1}$ and 13.01 to 19.67 $\mu\text{g mL}^{-1}$ in the presence and absence of L tryptophan, respectively. The Isolate SM4 showed maximum IAA production in the presence of L-TRP (45.3 $\mu\text{g mL}^{-1}$) or without LTRP (19.67 $\mu\text{g mL}^{-1}$). The isolates produced ACC in the range of 16–29 $\mu\text{mol/g}$. The maximum ACC concentration was observed by isolate SM4 (29 $\mu\text{mol/g}$) followed by SM1 (24 $\mu\text{mol/g}$).

Table 1. Phenotypic, growth and biochemical characterization of seed endophytic bacteria.

	SM1	SM4	SM8	SM9	SM14	SM19	SM21	SM23	SM24
Phenotypic characterization									
Colony color	Yellow	White	Yellow	White	Yellow	Yellow	White	White	Yellow
Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Size (mm)	2.5	3.1	2.1	1.8	3.0	3.0	2.6	4.0	4.0
Gram reaction	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Bacterium shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Growth conditions									
NaCl									
0.5%	++	++	+	+	++	++	++	++	+
1.0%	++	++	+	+	++	++	+	++	++
1.5%	++	+	+	+	+	++	+	++	++
2.0%	++	++	+	+	++	+	+	++	+
2.5%	++	++	++	+	+	+	+	+	+
pH									
4	+	+	-	-	+	+	+	+	-
5	+	+	+	+	+	+	+	+	-
6	+	+	+	+	+	+	+	+	+
7	+	++	+	+	+	+	+	+	+
8	+	++	+	+	+	+	+	+	+
9	+	+	+	-	+	+	+	+	+
Biochemical Characterization									
P solubilization	+	+	+	+	-	+	+	+	-
Siderophore									
Production	+	+	+	-	-	-	+	-	-
Catalase activity	+	+	+	+	+	+	+	+	+
Chitinase activity	-	+	+	+	+	+	+	-	-
Oxidase activity	+	+	+	+	+	+	-	+	-
Exopolysaccharide									
production	-	+	+	+	+	+	+	+	-
ACC deaminase ($\mu\text{mol/g}$)	24 ± 0.35	29 ± 0.68	18 ± 0.21	21 ± 0.35	24 ± 0.35	21 ± 0.52	18 ± 0.71	23 ± 0.42	16 ± 0.76
Auxin production (IAA equivalents $\mu\text{g mL}^{-1}$)									
With L-TRP	39.4 ± 0.08	45.3 ± 0.05	25.9 ± 0.18	32.9 ± 0.12	28.5 ± 0.04	29.4 ± 0.19	24.9 ± 0.12	35.4 ± 0.22	28.3 ± 0.09
Without L-TRP	17.3 ± 0.14	19.7 ± 0.21	14.9 ± 0.12	14.2 ± 0.18	13.0 ± 0.09	15.3 ± 0.22	14.4 ± 0.09	15.7 ± 0.15	14.2 ± 0.26

-, No activity; +, moderate efficiency; ++, high efficiency.

Plant growth parameters of maize seedlings were significantly increased by the inoculation of seed endophytic bacterial isolates than uninoculated control seedlings under stressed and unstressed conditions. The inoculated seedlings of maize recorded 7–45% and 13–51% higher shoot length under normal and stressed conditions, respectively than the respective control (Figure 2a). Similarly, root length was increased 10–60% and 15–54% under a normal and stressed condition, respectively (Figure 2b). A corresponding increase in seedling fresh and dry biomass was also found by the seed endophytic bacterial inoculation over control. The increase in fresh and dry biomass was recorded 13–60% and 5–121% under normal conditions and 10–51% and 10–167% under drought stress, respectively (Figure 2c,d). Among the positive effect of all the isolates, the isolates SM1, SM4, SM19 and SM23 exhibited statistically significant ($p < 0.05$) results in plant growth related attributes. However, the isolates SM8, SM9, SM14, SM21, and SM24 did not display much more vigorous growth in both conditions as compared to a non-inoculated control plant. Moreover, endophytic inoculation significantly increased the leaf area under both conditions (Figure 3a). The isolate SM4 resulted in 30% under normal and 49% higher leaf area under stress conditions over respective control.

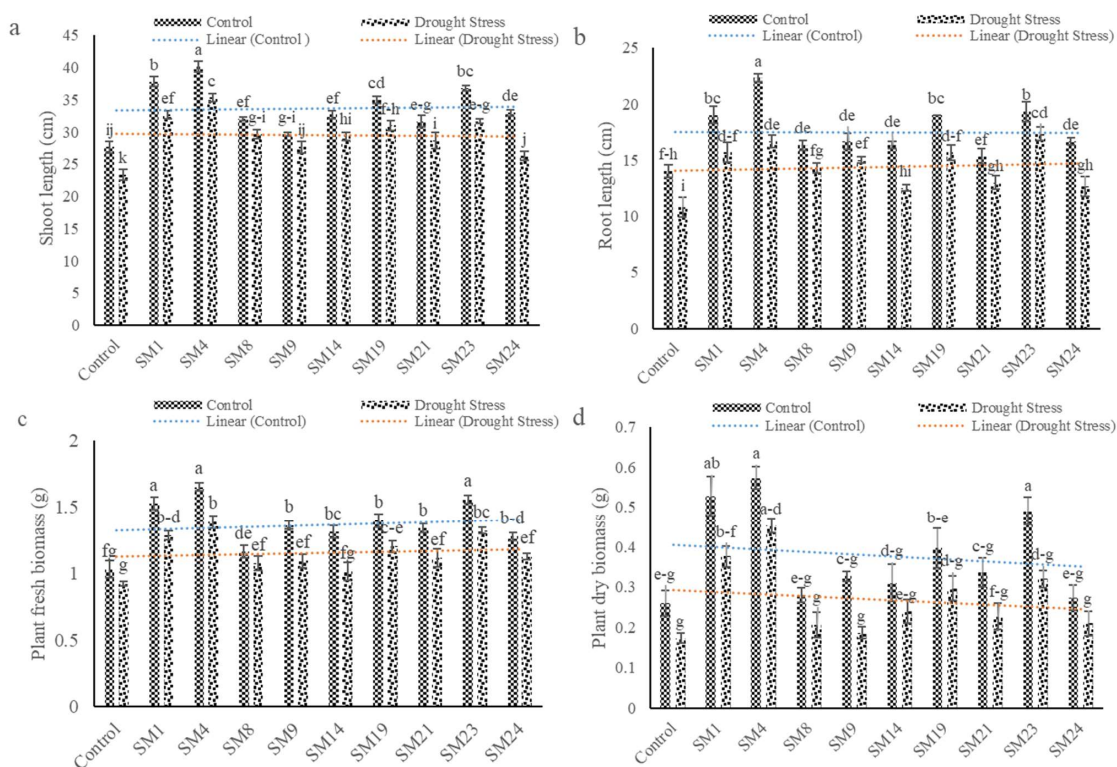


Figure 2. Effect of seed endophytic bacteria inoculation on shoot length (a), root length (b), plant fresh biomass (c) and plant dry biomass (d) of maize seedlings at different moisture levels under axenic condition ($n = 3$).

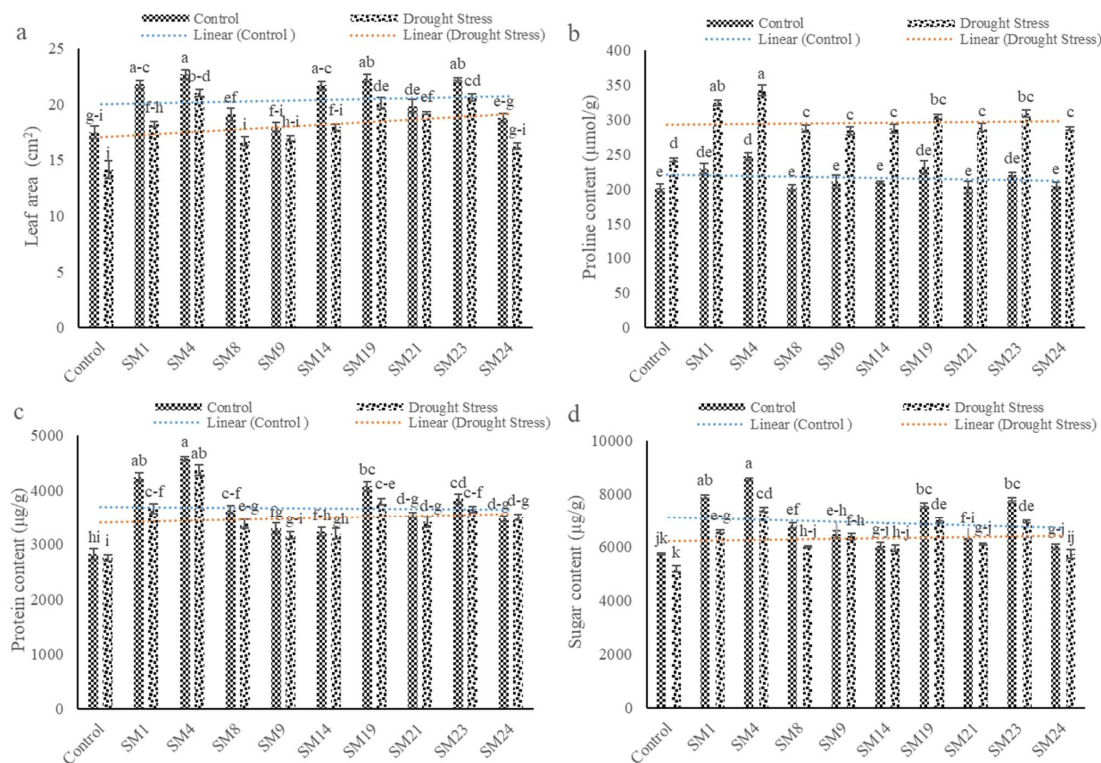


Figure 3. Effect of seed endophytic bacteria inoculation on leaf area (a), proline content (b), protein content (c) and sugar content (d) of maize seedlings at different moisture levels under axenic condition ($n = 3$).

The effect of seed endophytic bacteria inoculation on biochemical attributes of maize seedlings was determined by estimation of proline, protein and soluble sugar content (Figure 3b–d). The seed endophytic inoculation significantly increased the proline content over uninoculated control. The maximum increase in proline content was found by isolate SM4 followed by SM1 which was 23% and 16% higher than control under water deficit conditions, respectively (Figure 3b). Under normal irrigation, bacterial inoculation did not show significant results between inoculated and non-inoculated plants. In the case of protein and sugar content, all isolates positively increased their concentration as compared to control in both conditions (Figure 3c,d).

Drought stress significantly increased the antioxidant activity in uninoculated control seedlings but the bacterial inoculation showed a remarkable decrease in CAT, GPX, GR and SOD content and play a role to tolerate drought stress (Figure 4). Under normal condition, there was a non-significant difference in GPX and GR activity were observed between all the treatments and control plant. However, inoculated seedlings showed significant results of CAT and SOD activity under normal and stressed conditions. The isolates SM1, SM4, SM19 and SM23 were found most prominent in decreasing antioxidant enzyme activity. The isolate SM4 showed a maximum decrease in CAT, GPX, GR and SOD activity with a decrease of 72, 47, 49 and 26% over uninoculated control under water deficit conditions, respectively.

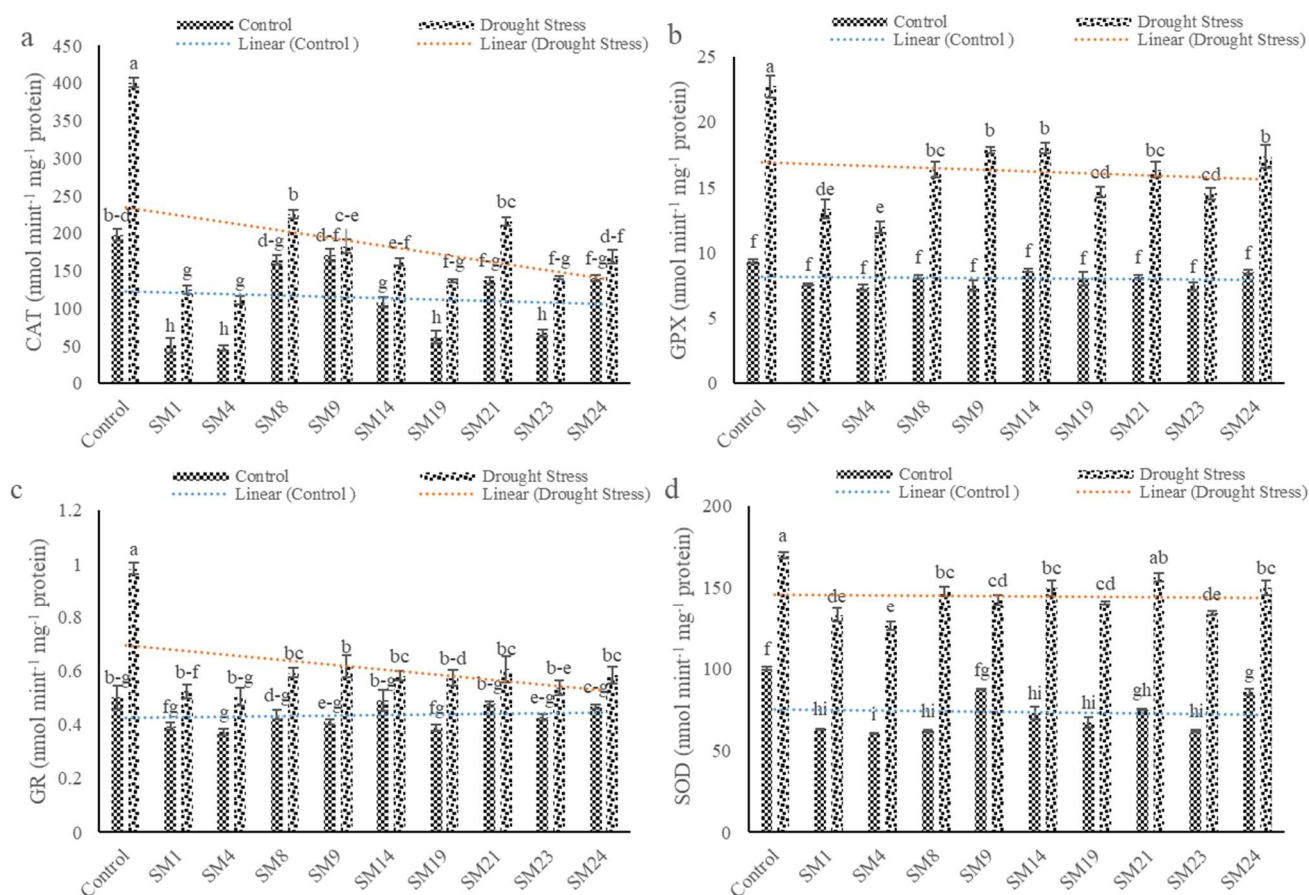


Figure 4. Effect of seed endophytic bacteria inoculation on Catalase (a), glutathione peroxidase (b), glutathione reductase (c) and super oxide dismutase (d) of maize seedlings at different moisture levels under axenic condition ($n = 3$).

Pearson's correlation analysis was conducted to investigate the relationship between growth parameters, biochemicals, and antioxidant activity in normal and stress conditions. For the formation of heatmaps, these factors were primarily focused and assembled as one unit (Figure 5a,b). Furthermore, Pearson's correlation coefficient of the above mentioned parameters were gathered into two groups. Group 1 contained growth parameters and biochemicals attributes, whereas group 2 contained antioxidant activity. Growth parameters and biochemical attributes shared a significant positive correlation under both conditions. In contrast, significant negative correlations were observed between group 1 and group 2. On the other hand, the correlation values within group 2 were strongly positively correlated.

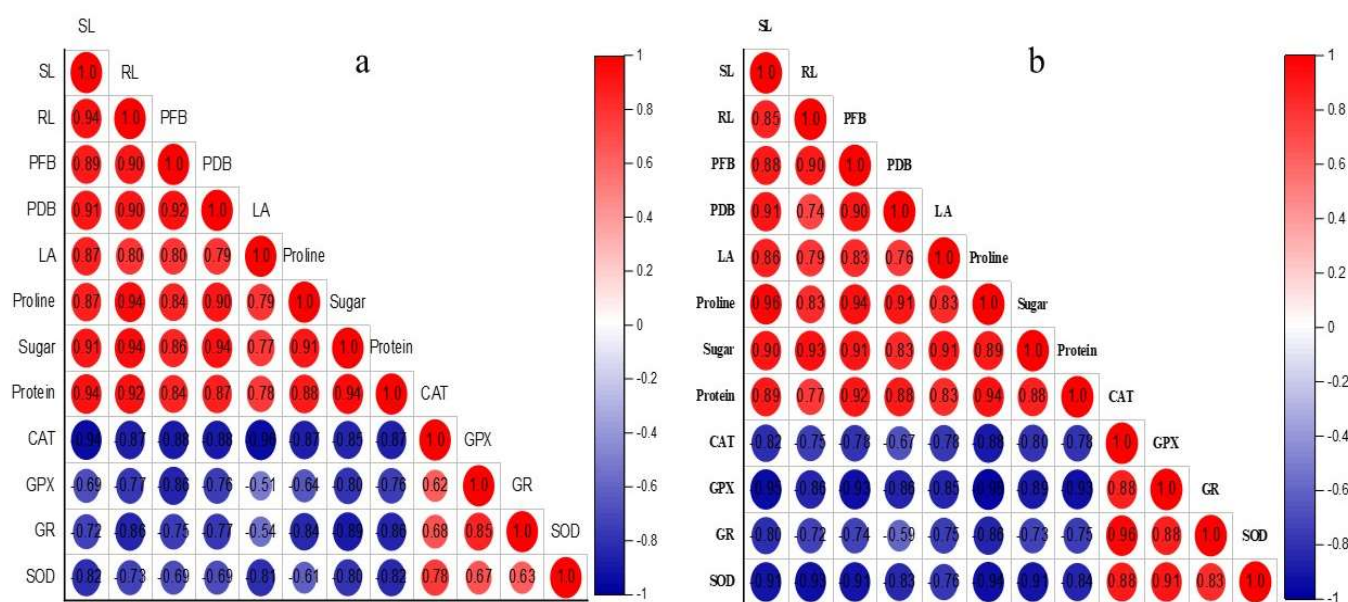


Figure 5. Represents correlation among measured parameters of maize under control (a) and drought stress (b). Abbreviations are as Shoot Length (SL), Root length (RL), Plant Fresh Biomass (PFB), Plant Dry Biomass (PDB), Leaf Area (LA), Catalase (CAT), Glutathione Per Oxidase (GPX), Glutathione Reductase (GR) and Super Oxide Dismutase (SOD).

Principle component analysis (PCA) was performed to further investigate the relationship and influence of different treatments (Figure 6a,b). The PCA was constructed using growth metrics, biochemical, and antioxidant activity as response variables. These results indicated that all 10 treatments were significantly separated by different components under observation, suggesting that treatment distribution is more sensitive to bacterial strains. This notion is also strengthened by Pearson's correlation values. Therefore, we conclude that the application of different bacterial strains had a differential effect on response variables.

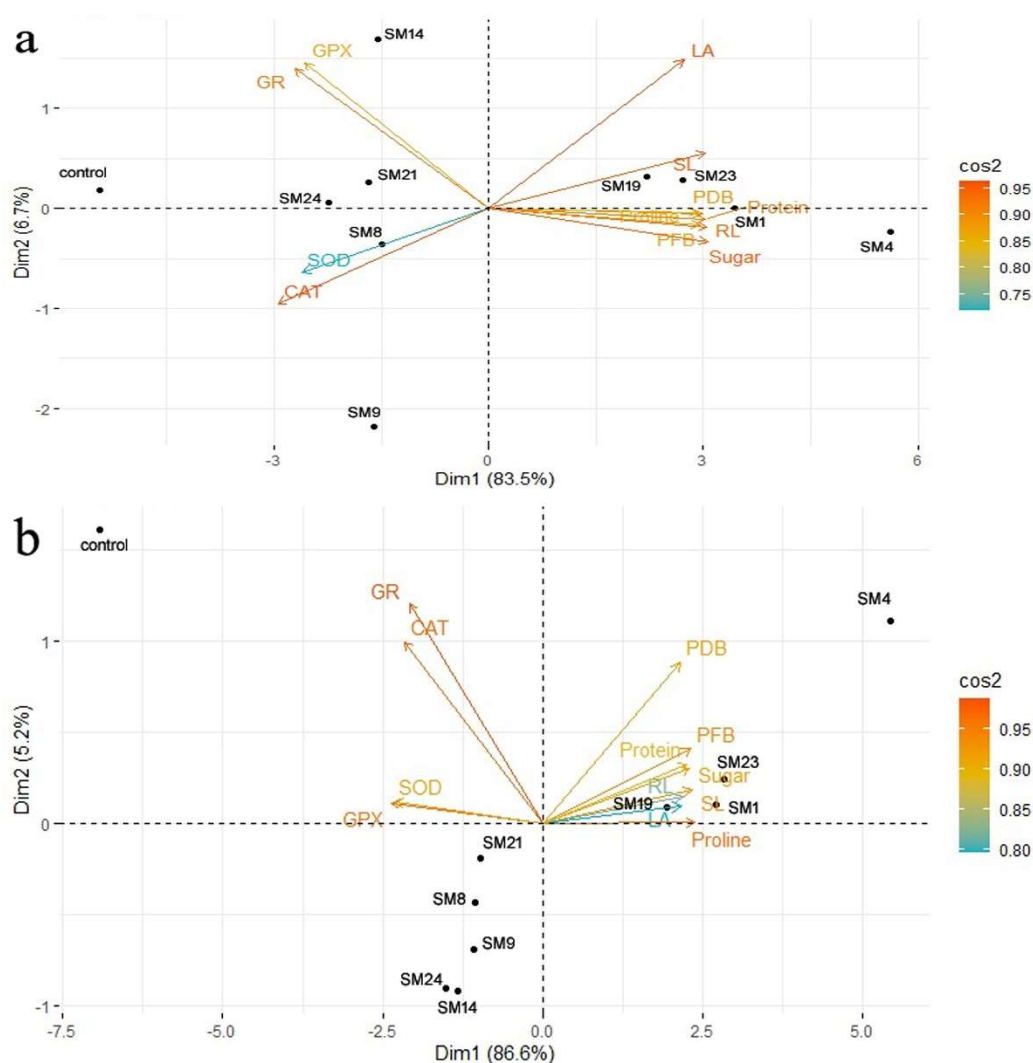


Figure 6. PCA-Biplot showing the relationship between the bacterial isolates and different attributes of maize in control (a) and drought stress (b). Abbreviations are as Shoot Length (SL), Root length (RL), Plant Fresh Biomass (PFB), Plant Dry Biomass (PDB), Leaf Area (LA), Catalase (CAT), Glutathione Peroxidase (GPX), Glutathione Reductase (GR) and Super Oxide Dismutase (SOD).

4. Discussion

The endophytic bacterial isolates from the maize seeds showed survival at different water potential i.e., 10, 20, and 30% PEG levels which were equal to -0.18 , -0.491 , and -1.025 MPa osmotic potential, respectively (Figure 1). Endophytic bacteria *Streptomyces* sp. have been shown to survive -0.05 to -0.73 MPa and stimulate wheat growth [6]. Similar to the current study, it has been reported that endophytes can tolerate up to -1.02 MPa of water deficit conditions and promote disease resistance [37]. Findings of another study revealed that *Pseudomonas* spp. have been observed to tolerate abiotic stresses (e.g., temperature, salinity, and drought) [55,56]. Several processes, such as the development of polymeric compounds typically called exopolysaccharides, are involved in their survival under high water stress levels, which are secreted into the micro-environment outside the cells where they interact with a plant (rhizosphere). These exopolysaccharides keep the bacterial cells hydrated and provide carbon and nutrients under drought stress. These saccharides also help plants indirectly by aggregation of soil particles which increase water and nutrient holding capacity by improving soil structure [57]. The synthesis of osmolytes, stress proteins, and changes in cell morphology are also involved in stress tolerance to preserve

cell potential, prevent cell disruption and adopt a stress environment [58]. The catalase and oxidase production help to maintain metabolism and prevent membrane damage by reducing H_2O_2 [59].

In this study, several isolates produced IAA and ACC deaminase enzymes, which are important for plant growth promotion (Table 1). Many endophytes producing IAA have also been documented to exhibit sensitivity towards endogenous hormones and are able to cope with drought stress [60]. IAA produced in plants by endophytes increases root length, promotes nutrient access, and enhances root exudation, providing more opportunities for soil microbes to communicate with roots [61]. A large number of auxin-responsive genes have been differentially expressed under different conditions of abiotic stress. The role of auxin in plant defense mechanisms and growth promotion during stress demonstrates the significance of auxin under stress conditions [62]. Likewise, it is convincingly presumed that the role of IAA is also a standard in a stressed environment [63]. In addition, bacterial endophytes produce siderophores and solubilize soil phosphorus when initiating symbiotic relationships with host plants [64]. Siderophores are organic compounds secreted in iron-limited conditions by microorganisms and plants that enable microbial and plant cells to chelate iron from the environment for uptake [65].

Similarly, immobile phosphorus in soil is solubilized by microorganisms, an essential feature for plant growth promotion [66]. The processes and significance of phosphorus solubilizing microorganisms in agriculture have been illustrated in several recent reviews [64,67]. The capability of endophytic bacteria to sequester ACC and probably degrading it for the provision of energy and nutrients (nitrogen) to the host plant is been reported. The bacteria can efficiently counter the harmful effects of ethylene by ACC activity and thus plant growth increases substantially [68]. Therefore, endophytic bacteria producing ACC deaminase play a substantial role to increase the growth attributes of several vegetables under water deficit conditions [2]. Thus, endophytic bacteria are key drivers to increase the quantitative attributes of plants and the accumulation of nitrogen in seeds, which is the first step to restore nodulation under water deficit conditions. It is observed that bacterial inoculation reduces water deficit stress in pea plant and increase growth and yield [69].

The remarkable increase in shoot length, root length, leaf area, seedling fresh biomass, and seedling dry biomass was observed by inoculation of seed endophytic bacteria concerning control plants under normal and drought conditions (Figure 2). The substantial increase in plant growth was due to plant growth attributes of seed endophytic bacteria. The use of endophytic bacteria for plant growth promotion has previously been documented in several studies. The increase in shoot dry biomass, stalk length, and leaf area has been shown by the inoculation of *Bacillus* sp. to maize seedlings [70]. Similarly, in an observation, plant growth-promoting effects were found by *Bacillus* sp. [71]. In the selected isolates, phosphorous solubilization ability was also present, which was useful for improving nutrients available to plants. Biofilm formation around plant roots due to the production of exopolysaccharides by endophytic bacteria is also helpful for the channeling of nutrients and water to plants under dry conditions. In the current study, maize plant growth promotion is partially linked to the release of exopolysaccharide, IAA, ACC deaminase, and nutrient solubilization by the application of isolates.

The inoculation of seed endophytic bacteria significantly increased the protein content and soluble sugar in leaves (Figure 3). Under abiotic stress, increased protein concentration prevents the breakdown and denaturation of cellular components [72]. The importance of soluble sugars has been reported in the literature, and it has also been highlighted that these osmolytes play a remarkable role in plants' survival under drought stress. Inoculated seedlings with *Pseudomonas* sp. improved soluble sugar content as compared to uninoculated seedlings, indicating that bacteria hydrolyze starch content, and then additional sugar was made accessible for osmotic adjustment to mitigate stress. Moreover, the negative effects of water deficit conditions on uninoculated plants are due to decreased starch and sugar levels [73].

The plants treated with endophytic bacteria showed an increase in proline content under water deficit conditions over untreated plants (Figure 3). The role of proline content to counter environmental stress especially water stress is been investigated for several decades. Accumulation of proline by various plants is a strategy to maintain osmotic turgor pressure under stressed conditions [74]. Moreover, in literature, it is suggested to stabilize macromolecules, act as a carbon and nitrogen sink for use after stress relief, aid in free radical detox [75]. Plants inoculated with *Pseudomonas* sp. improved proline content under water stress. This may be linked to the upregulation of the proline biosynthetic channel to maintain proline at a higher concentration, which has helped maintain the cell's water state and protect membranes from stress due to drought [76].

In addition, the inoculated endophytes have produced osmolytes and antioxidants which are also responsible to strengthen the drought tolerance of plants. In the present study, drought stress increased the concentration of the antioxidant enzymes i.e., Proline, CAT, GPX, GR, and SOD in the maize seedlings. The maize plants inoculated with seed endophytic bacteria revealed a maximum decrease in the antioxidant enzyme activity under drought stress (Figure 4). Interestingly, there is a major interaction between antioxidant enzymes and drought stress, but inoculation with PGPR decreased the detrimental effect of drought stress [77]. In the event of drought stress, most of the damage takes place at a cellular level due to an imbalance production of ROS. Stress proteins are water-soluble and have a role in tolerating stress [78].

5. Conclusions

In conclusion, this study investigated the potential benefits of seed endophytic bacterial isolates from maize seeds. Results revealed that drought has a detrimental effect on maize (*Zea mays* L.) growth and physiology. Drought-stressed plants benefit from seed endophytic bacteria inoculation, which helped them to maintain normal development. Drought tolerance in maize was improved by endophytic bacteria inoculation through a variety of mechanisms, including phosphate solubilization, siderophore production, and exopolysaccharides. Among all bacterial isolates, SM1, SM4, SM19 and SM23 performed better. Inoculation with seed endophytic bacteria may be a successful approach for promoting maize growth and physiology under drought stress. The results of this experiment encourage to collect more reliable data for further investigation of these isolates in field scale under drought conditions and the potential strains might be used to create bio-inoculants for critical rain-fed crops. Furthermore, the molecular mechanisms behind microorganism-induced abiotic stress tolerance in plants must be investigated.

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